SUBSITITUTE PATENT SPECIFICATION

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PREPARATION METHOD OF VALIENAMINE FROM ACARBOSE AND/OR ACARBOSE DERIVATIVES USING TRIFLUOROACETIC ACID

CROSS-REFERENCE TO RELATED APPLICATIONS

[01] This application claims the benefit of PCT International Application Number PCT/KR2002/002198 under 35 U.S.C. §371.

BACKGROUND OF THE INVENTION

[02] **Field of the Invention**

[03] The present invention relates to a method of producing valienamine and, more particularly, to a method of producing valienamine at a substantially high conversion rate. The method involves mass producing valienamine by way of selective hydrolysis by using TFA from acarbose or acarbose derivatives, and then removing its by-products therefrom, i.e., monosaccharides, disaccharides, and trisaccharides.

[04] **Brief Description of the Related Art**

[05] The conventional art relating to commercial production of valienamine can be divided into two types. The first type relates to a method of direction production of valienamine by using microorganism fermentation, and the second type relates to a method of production of validamycin, a derivative of valienamine, by degradation by using other microorganisms.

[06] Validamycin derivatives basically include a valienamine moiety which selectively binds with validamine or valiolamine. Moreover, a validamycin derivative is a pseudotrisaccharide compound which is glucose bonded in chain.

[07] The validamycin compound is an antibiotic used for germicide for rice-cultivated land in East Asia which is produced among other methods by culturing *Streptomyces hygroscopicus*, a soil microorganism. Here, the validamycin compound contains a small amount of intermediate valienamine which is then separated out via a column.

[08] As for another method for producing valienamine, there is a method of separating validamycin by using a microorganism, *F. saccharophilum*, etc. The method involves using validamycin as a substrate or medium and adding it to the liquid mixed with microorganisms; culturing them for a certain period of time; and then inducing separation of validamycin by microorganisms; and then obtaining validamycin by separation via a column. Yet, the two methods have disadvantages in that they take too much time for microorganism fermentation with not much higher yield.

[09] Another compound which has a valienamine moiety is acarbose. Acarbose is obtained from secondary metabolic products of *Actinoplanes sp.*, which is one type of soil microorganisms. It is currently being used as a treatment for diabetes, since it has inhibition effects on α-amylase. However, as of yet, there is no disclosure of the process of commercial or mass production of valienamine by using acarbose as a raw material.

[10] As for methods of producing valienamine, reported in academia, there is a chemical method of producing valienamine by using N-bromosuccinimide (NBS) with validamycin as raw material. However, as this method uses dimethyl sulfoxide (DMSO) as solvent, it suffers from difficulties during

purification and separation processes of byproducts, in addition to its low yield. Moreover, there is an ongoing research into the production method of valienamine using organic and inorganic acid, such as sulfuric acid, hydrochloric acid, and acetic acid. Yet, the method is not practical since it is limited to the extent of hydrolysis of only one terminal saccharide. Moreover, there was an attempt to produce valienamine by way of organic synthesis, but it currently is in a standstill due to the inefficiency of purification and organic synthesis processes.

[11] There is a production method of valienamine by pre-synthesis by enzyme, which is being actively pursued in recent years. This is a method of producing valienamine by using an inexpensive substrate by finding valienamine synthesis and related enzymes expressed by a strain. However, there are many difficulties, such as determination of the degree of activity and the expression according to a gene probe. So, the current production is rather difficult.

[12] As stated above, the production of valienamine *in vitro* by purification enzymes or chemicals has not yet been commercialized, and so up to now, valienamine has been mainly synthesized and produced by hydrolyzing validoxylamine and validamycin by using the strains, *Pseudomonas denitrificans* and *Flavobacterium saccharophilum*. Japanese Patent No. 57,054,593 discloses a reaction of converting validoxylamine and validamycin by using microorganisms. This is a method of synthesizing and producing valienamine by using *Flavobacterium saccharophilum* by reacting 1-5 wt.% mixture of validoxylamine and validamycin for about 24 to about 200 hours at reaction conditions of 20-45°C and pH 5-8.

SUMMARY OF THE INVENTION

[13] The present invention purports to provide a method of producing valienamine at a substantially high conversion rate. The method involves first mass producing valienamine through selective hydrolysis from acarbose or acarbose derivatives by using TFA, and then removing the byproducts, i.e., monosaccharides, disaccharides, and trisaccharides.

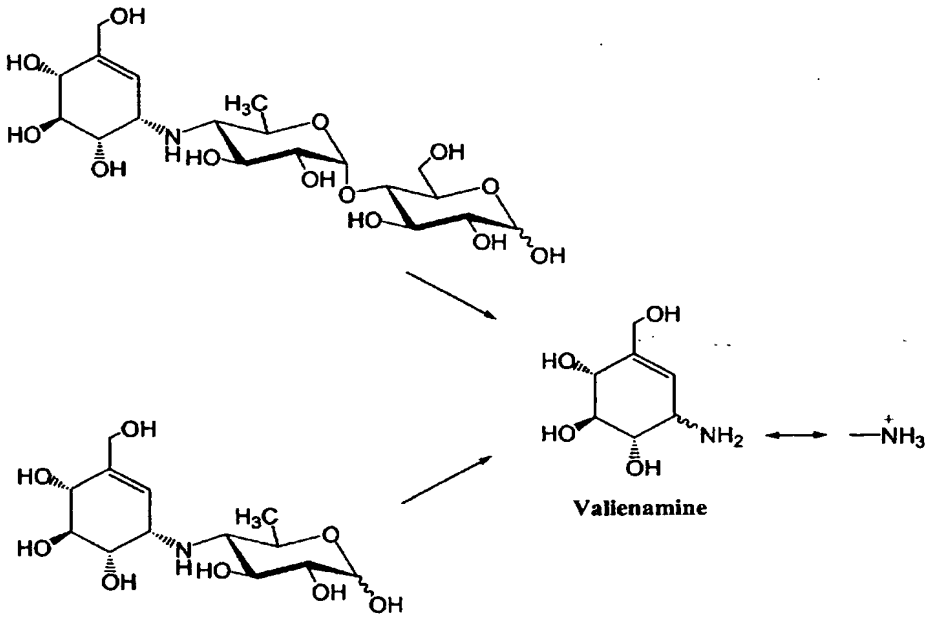
[14] To achieve said objectives, the present invention involves a method of producing valienamine from acarbose or acarbose derivatives by using trifluoroacetic acid (TFA). In particular, the present invention provides a method of producing valienamine by using a reaction substrate of final concentration of 0.2-10% acarbose or acarbose derivatives, and a reaction solvent of 10-60% TFA solution.

[15] If the final concentration of acarbose or its derivatives is less than 0.2%, or that of TFA exceeds 60%, the production cost per unit increases. On the other hand, if the final concentration of acarbose or its derivatives exceeds 10%, or that of TFA is less than 10%, the yield therein decreases.

[16] Moreover, the present invention provides a method of producing valienamine from acarbose or acarbose derivatives by using TFA, which is characterized by reacting it for about 1 to about 24 hours at about 80 to about 120°C, or by using a high-temperature and high-pressure autoclave, which can reduce reaction time to one hour and increase its yield up to 96%.

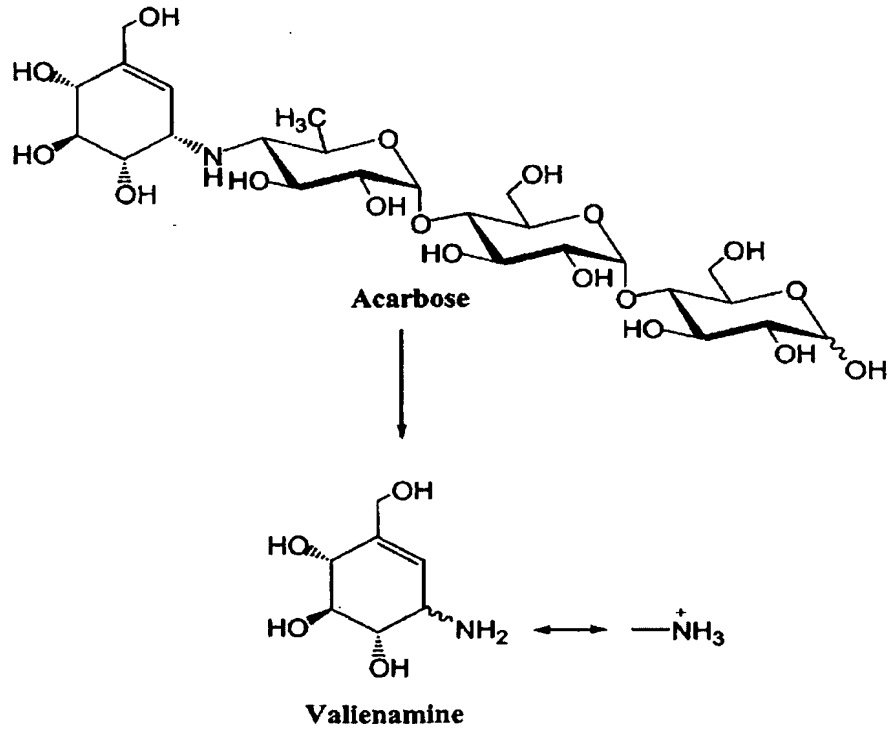
[17] Accordingly, the present invention can yield valienamine with an amine group of NH_2 or NH^+_3 at its carbon chain.

[18] **Formula 1**



[19]

[20] **Formula 2**



[21]

[22] Moreover, according to the present invention, an acarbose derivative is a compound having one, two, four, five or more saccharides bonded to a carbon chain, but generally refers to a derivative of one or two saccharides.

[23] Valienamine is known to have maltase and sucrase inhibition effects and to have antibiotic activity as against *Bacillus* species. Moreover, its intramolecular atom alignment is similar to that of alpha-D-glucose. The inhibition activity of alpha-glucosidase of valienamine is believed to be caused by structural similarity of valienamine to D-glucosyl cation. The D-glucosyl cation with an enzyme as a catalyst forms a half-chair conformation in a transition state, which is produced during hydrolysis of pyranoside.

[24] As for compounds with a valienamine moiety, there are acarbose, and its derivatives, validoxylamine, validamycin, etc. Among these, acarbose is being widely used as an inhibitor for Type II diabetics. Acarbose and acarbose derivatives have different structures from the other two compounds (validoxylamine and validamycin), and their production methods are substantially different as well.

[25] Compounds with a valienamine moiety, i.e., acarbose, and its derivatives, validoxylamine, and validamycin, are all potentially raw materials for valienamine. All of them are produced by fermentation by different bacteria strains, respectively. Among these, acarbose is being distributed as a diabetic treatment all over the world by a German pharmaceutical company, Bayer, Inc., along with Chinese and Japanese pharmaceutical companies. Acarbose is more expensive than validamycin but is easier to obtain in a pure raw material form. Accordingly, acarbose has the advantage of easy separation process, which makes it an appropriate raw material for valienamine. When using acarbose as a raw material, there is a problem of

difficult purification by way of pigments from cleaved saccharide, but this can be easily resolved by using acarbose derivatives.

BRIEF DESCRIPTION OF THE DRAWINGS

[26] The novel features that are characteristic of the present invention are set forth in the appended claims. However, the preferred embodiments of the invention, together with further objects and attendant advantages, will be best understood by reference to the following detailed description taken in connection with the accompanying drawings in which:

[27] Fig. 1 is a hydrogen NMR spectrum of valienamine produced from acarbose by using TFA;

[28] Fig. 2 is a carbon NMR spectrum of valienamine produced from acarbose by using TFA;

[29] Fig. 3 is a hydrogen NMR spectrum of valienamine produced from an acarbose derivative by using TFA;

[30] Fig. 4 is a carbon NMR spectrum of valienamine produced from an acarbose derivative by using TFA; and

[31] Fig. 5 is a graph of Bio-LC(HPLC) data of valienamine produced from an acarbose derivative by using TFA.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[32] The present invention is further illustrated by the following examples, but these examples should not be construed as limiting the scope of the invention

[33] **Example 1: Method Of Producing Valienamine Using TFA**

[34] 10 g of pure acarbose were place into 10% TFA solution at 5% final concentration. At a reaction temperature of 100°C, it was reacted for 12 hours or more, followed by removal of TFA and water. Then, by using ion-

exchange resins for purification, 2.02 g of valienamine were obtained respectively.

[35] **Example 2: Method Of Producing Valienamine Using TFA**

[36] 1 g of a pure acarbose derivative (monosaccharide and trisaccharide) were place into 10% TFA solution at 5% final concentration. At reaction temperature of 100°C, it was reacted for 12 hours or more, followed by removal of TFA and water. Then, by using ion-exchange resins for purification, 0.45 g and 0.31 g of valienamine were obtained, respectively.

[37] **Example 3: Method Of Producing Valienamine**

[38] **Using TFA By Using An Autoclave**

[39] 10 g of pure acarbose were place into 10% TFA solution at 5% final concentration. While putting pressure using an autoclave, at a reaction temperature of 121°C, it was reacted for 30 minutes to 1 hour, followed by removal of TFA and water. Then, by using ion-exchange resins for purification, 2.1 g of valienamine were obtained.

[40] **Example 4: Method Of Producing Valienamine**

[41] **Using TFA By Using An Autoclave**

[42] 1 g of a pure acarbose derivative (monosaccharide and trisaccharide) were place into 10% TFA solution at 5% final concentration. While putting pressure using an autoclave, at reaction temperature of 121°C, it was reacted for 30 minutes to 1 hour, followed by removal of TFA and water. Then, by using ion-exchange resins for purification, 0.46 g and 0.30 g of valienamine were obtained, respectively.

[43] The hydrogen and carbon NMR spectrums with respect to the resultant products obtained as a result of the reactions of Examples 1 and 2 are as follows: ^{1}H-NMR(D_2O) δ: 3.42(1H, br s, H-1), 3.54(2H, Abq, J=13.6Hz, H-7), 3.94(1H, d, J=6.79Hz), 3.97(1H), 4.05(1H), 5.64(1H, d,

J=4.6) ^{13}C-NMR(D_2O) δ: 48.9(C-1), 61.2(C-7), 69.7(C-2), 71.7(C-4), 72.0(C-3), 123.4(C-6), 139.9(C-5).

[44] By using the method of the present invention, valienamine can be produced from acarbose with a yield rate of about 50 to about 95%, or acarbose derivatives with a yield rate of about 70 to about 95%. Since hydrolysis is occurred on the α-binding adjacent to the amine moiety of valienamine, only monosaccharides, disaccharides, or trisaccharides are produced as byproducts. Due to this advantage, the refining process becomes simple making it possible to produce valienamine with high purity while reducing the pigments.

[45] In addition, by using the method of the present invention, the voglibose, which is widely sold as a remedial agent of diabetes over the world including Korea, Japan and China, can be more easily produced cutting down the production cost. Also the invention can contribute to the development of valienamine derivatives which have better pharmaceutical activity, or which can be used on other types of disease.

[46] It is appreciated by those skilled in the art that various changes and modifications can be made to the illustrated embodiments without departing from the spirit of the present invention. All such modifications and changes are intended to be covered by the appended claims.